

RESEARCH AND DEVELOPMENT, NEUCHATEL - QUARTERLY REPORT

DIVISION : RESEARCH
SUBJECT TITLE : TITANIA
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water-activity, ph, inhibition, activation,
heat-shock, cigarette, taste

OBJECTIVE

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To study risks of physiological changes in the bacterial population during tobacco processing and storage, and to investigate their impact on the organoleptic and chemical properties of tobacco.

STATUS

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The effect of the heat-shock on *Bacillus* spore germination was investigated as a function of water activity.

Cellular extracts of germinated spores were added to cut tobacco for analytical and subjective evaluation.

RESULTS

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Germination activation

In order to establish the impact of tobacco processing on *Bacillus* spore germination, the effects of heat-shock in combination with water activity (a_w) were evaluated in simulation trials. Simulation media were prepared from tobacco extract supplemented with glycerol to obtain a_w values ranging between 0.77 and 1.0 [1]. Spore suspensions were heat-shocked at 60, 70, 80, 90 and 100°C for 30 min. and cooled rapidly prior to the incubation in tobacco medium. Germination was followed over time by measuring the loss in absorbance at 660 nm [2].

Fig. 1 shows the effects of heat-shock temperature on spore germination for $a_w = 1.0$. At 90 and 100°C no germination could be observed. Compared to control the trials activated at 60, 70

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and 80°C show no lag phase at the beginning of germination. Completion of germination decreases by increasing activation temperature. After 90 min., 100% of the spore population had germinated at 60°C, 90% at 70°C and only 75% at 80°C. The germination rates follow the same trend. At 60 and 70°C the maximum rate measured was 2%/min. and at 80°C 1%/min. A germination rate of only 0.5%/min. was found in the control.

Fig. 2 shows combined effects of heat-shock and water activity after 120 min. incubation for five different a_w values. In the range of 0.9-1.0, spore germination is clearly activated by a heat-shock between 60 and 70°C compared to an unheated control. The version treated at 80°C shows the same profile as the control. At 90°C a slight germination (10%) was observed for a_w values of 0.9, but no germination was recorded for the activation at 100°C. The germination activation created by a heat-shock between 60 and 70°C and a water activity of between 0.9 and 1.0 corresponds to situations encountered during tobacco processing.

Germination and off-taste

A relation between *Bacillus* spore germination and a taste change in cigarettes was found [3]. It was assumed that activation conditions created in the dryer allowed spores to germinate and that germinated cells unable to outgrow on tobacco due to low a_w are osmolyzed. In order to verify this implication, a *B. pumilus* spore preparation was allowed to germinate, germinated cells were broken and cellular extract was injected into cigarettes for subjective evaluation. Two versions were prepared: one with the equivalent of the tobacco microflora (i.e. 10^6 cells/g) and the other with ten times more cell extract. After conditioning, the two versions, as well as a water treated control were evaluated vs. an untreated control by an in-house panel.

Version	Comments	Preference
untreated control	standard quality	1
control + water	idem control	1
+ 1 cell. equival.	less flavor sensations, slight dark side	3
+ 10 cell. equival.	after taste, cover the mouth, dark side	4

Preferences : 1 = best 4 = worst

Both controls were in line with the standard quality. An increase in taste change was recorded with increasing cellular extract concentration. The main line in the taste description concerns a deviation to the dark side. This dark note was also described by an expert panel for off-taste cigarettes from project PLEIADE [3,4].

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CONCLUSIONS

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Heat-shock temperatures of 60 and 70°C in the aw range of 0.9-1.0 considerably accelerate *Bacillus pumilus* spore germination.

Cellular extract of germinated spores added to cut filler causes off-taste in cigarettes.

PLANS

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- Study germination activation by heat-shock and aw during Burley and cut tobacco treatments.
- Produce at mini-primary scale a cut filler sprayed with cellular extract of germinated spores for subjective and chemical evaluation.
- Verify the impact of cellular extract on cigarette taste by panel A evaluation and establish a correlation with known off-taste problems.
- Collaborate on chemical investigations on cellular extract composition and the possible interactions with the tobacco matrix.

REFERENCES

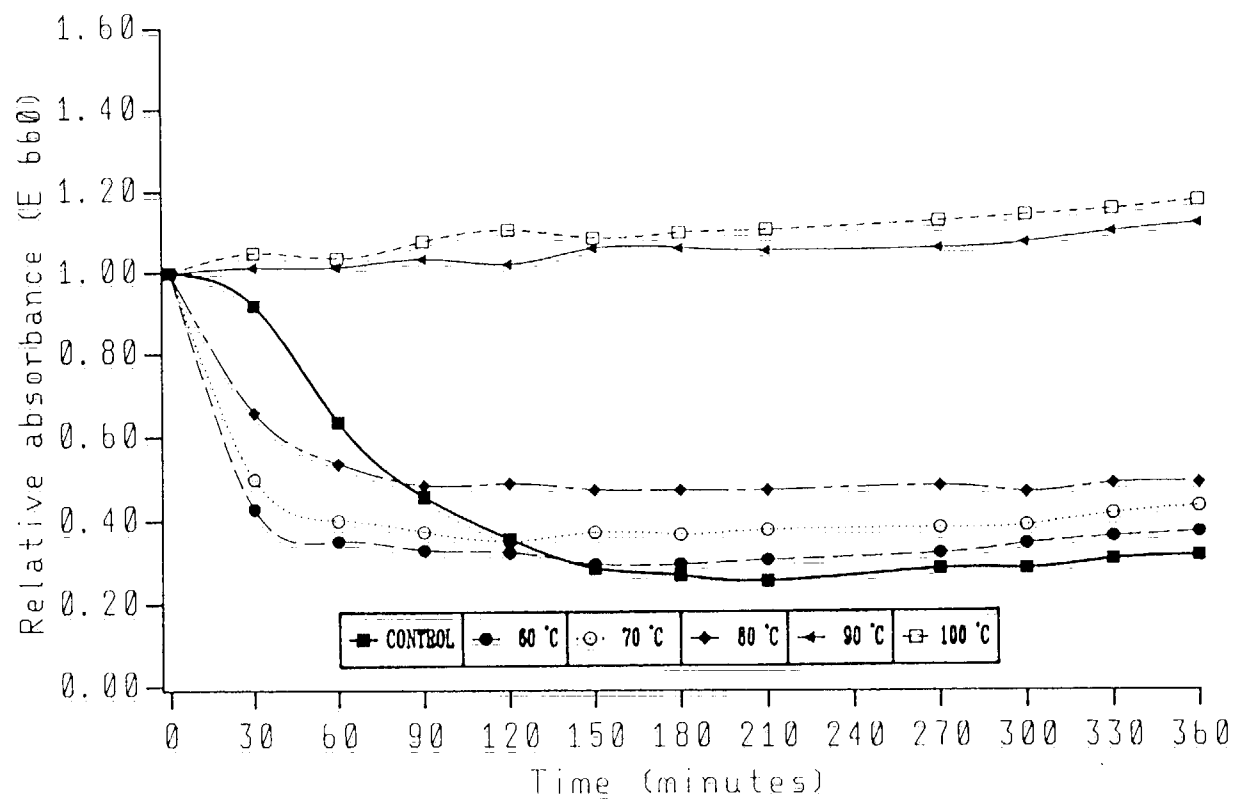
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- [1] Hofer-M., Kälin-P., Quarterly Report, EUROP, January-March 1988.
- [2] Kälin-P., Quarterly Report, EUROP, April-June 1988.
- [3] Memo from Hofer-M. to Fink-W., Project PLEIADE I, July 14, 1988.
- [4] Memo from Hofer-M. to Fink-W., Project PLEIADE II, November 22, 1988.

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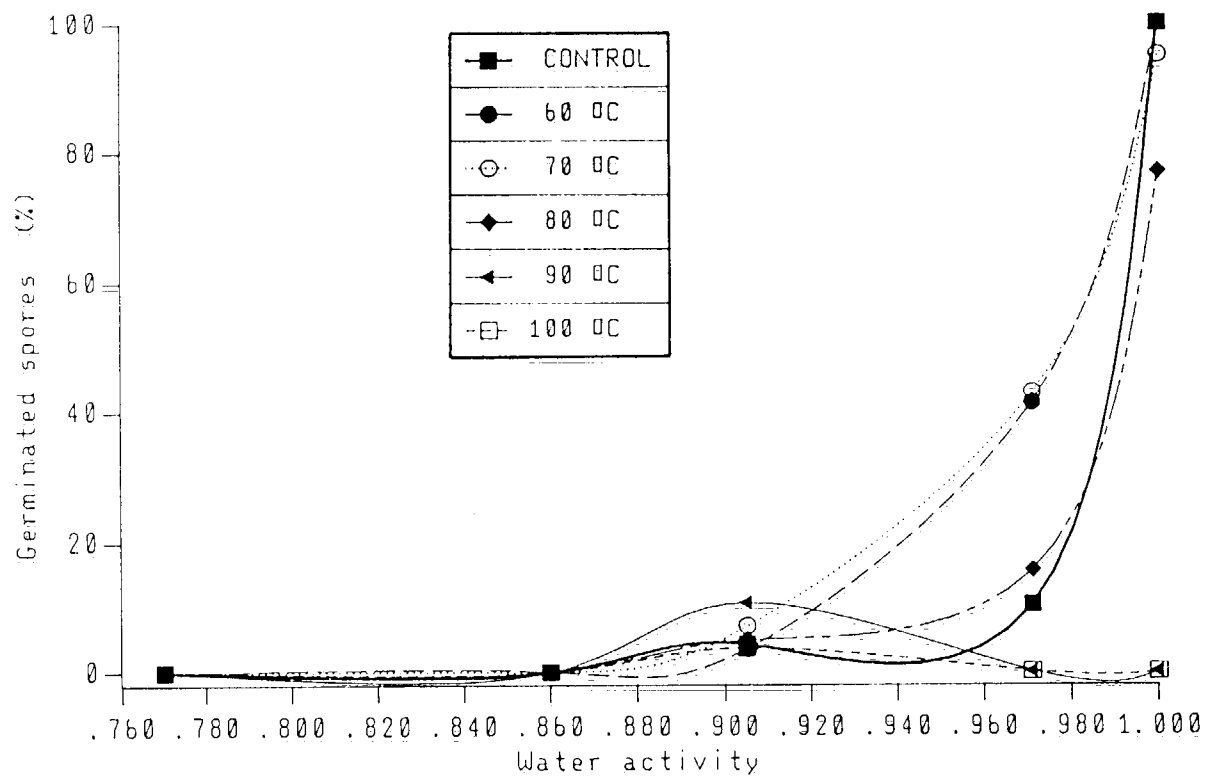
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Figure 1 : EFFECT OF HEAT-SHOCK ON SPORE GERMINATION
AW = 1



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Figure 2 :

EFFECT OF HEAT-SHOCK ON SPORE GERMINATION
AS A FUNCTION OF WATER ACTIVITY

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